

with phosphate buffered saline. As used in this disclosure, all column dimensions are expressed as diameter in centimeters \times height in centimeters. Antiserum (10 mL of Cappel α -digoxin serum at 4.5 mg/mL monospecific antibody) was applied at a flow rate of 1 mL per minute. The column was washed with phosphate buffered saline until the absorbance at 280 nm reached baseline. Antibody was then eluted from the column with 60 mL of 3 M NH_4SCN (pH 7.5) and immediately dialyzed against 4 \times 2 L changes of phosphate buffered saline at 4 $^\circ$ C.

(C) Digoxin Assay

Measurement of sample digoxin was performed in the following manner: a ouabain-affinity column as prepared in (B) above (0.5 cm \times 8 cm) was loaded with a solution of fluoresceinated affinity-purified antibodies (10 mL at 0.4 mg/mL) and then washed with 50 mL of phosphate buffered saline containing 1 mg/mL HSA. Samples (500 μ L) containing various amounts of digoxin in a solution of phosphate buffered saline containing HSA were percolated through the column followed by 4.5 mL of the same albumin-buffer solution at a flow rate of 0.5 mL per minute. The fluorescence in the eluates was then measured in an Aminco Spectrofluorometer and plotted as a function of the digoxin concentration in the original sample. The results are shown in FIG. 1.

EXAMPLE II

Enzyme Immunoassay for Digoxin

The antibody reagent used in this example was a conjugate prepared by binding the monovalent antibody fragment Fab' to β -galactosidase using the heterobifunctional reagent m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS).

(A) PREPARATION OF Fab' β -GALACTOSIDASE CONJUGATE

Digoxin-specific antibodies were immunopurified directly from whole rabbit serum using the following representative protocol: Ouabain affinity resin was packed into a column (0.7 cm \times 15 cm) to a bed volume of 6 mL and equilibrated with phosphate buffered saline. Antiserum (10 mL of Cappel α -digoxin serum at 4.5 mg/mL monospecific antibody) was applied at a flow rate of <1 mL per minute. The column was washed with phosphate buffered saline until the absorbance at 280 nm reached baseline (<0.01). Antibody was then eluted from the column with 60 mL of 3 M NH_4SCN (pH 7.5) and immediately dialyzed against 4 \times 2 L changes of phosphate buffered saline at 4 $^\circ$ C.

Fab' fragments were prepared from affinity purified antibodies via pepsin digestion. Twenty mL of affinity-purified α -digoxin antibodies was concentrated to 2 mL on an Amicon stirred-cell apparatus (PM-30 membrane). The final protein concentration was measured to be 10 mg/mL. The sample was dialyzed against 1000 mL of 0.1 M sodium acetate, pH 4.5, for 4 hours at 4 $^\circ$ C. After dialysis, 20 μ L of a 10 mg/mL solution of pepsin, dissolved in the same sodium acetate buffer, was added and the temperature raised to 37 $^\circ$ C. for 20 hours. After this digestion period, the sample was clarified by a brief centrifugation and then chromatographed on a Sephadex G-150 (cross-linked, beaded dextran having 150,000 dalton exclusion limit) column (1.5 cm \times 90 cm) equilibrated in 0.015 M sodium phosphate (pH 7.4), 0.15 M NaCl (phosphate buffered saline). The column fractions containing the (Fab')₂ fragments, identified by gel electrophoresis, were pooled (19.2 mL) and then concentrated to 2.0 mL by pressure filtration (PM-30 Amicon

membrane). After concentration, the (Fab')₂ fragments were reduced to their corresponding Fab' fragments by adding 40 μ L of a 1 M dithiothreitol solution. The reduction was performed at 25 $^\circ$ C. for 90 min under argon. The Fab' fragments were then reacted with 14.8 mg of iodoacetamide at 25 $^\circ$ C. for 2 hours under argon. Reaction products were removed by dialysis at 4 $^\circ$ C. against 3 \times 4 liters of phosphate buffered saline.

The Fab' fragments so produced were then reacted with a 20-fold molar excess of MBS. Eighty-five microliters of a 79 mM solution of MBS in tetrahydrofuran was added to the 2 mL solution of Fab' fragments and reacted for 1 hr. at 25 $^\circ$ C. under argon. The mixture was desalted on a Sephadex G-25 (cross-linked, beaded dextran having 5000 dalton exclusion limit) column (1.5 cm \times 40 cm) in phosphate buffered saline. The derivatized Fab' fragments, which eluted in the void volume, were pooled and combined with 2 mL of β -galactosidase at 12 mg/mL in phosphate buffered saline at 4 $^\circ$ C. After 16 hours, this solution was concentrated to 2 mL on an Amicon PM-30 pressure filtration stirred-cell followed by column chromatography on Sepharose 4B-CL (cross-linked, macroporous agarose in bead form having 1-5 \times 10⁶ dalton exclusion limit in a 1.5 cm \times 90 cm column). The Fab'- β -galactosidase conjugate was eluted with the free β -galactosidase. The entire peak of enzyme activity was pooled and subsequently immunopurified on the ouabain affinity column. The procedure for immunopurification was as follows: Pooled column fractions from the Sepharose 4B-CL column were eluted through the ouabain affinity column (1.0 cm \times 7.0 cm), followed by 100 mL of phosphate buffered saline. The Fab'- β -galactosidase conjugate was then eluted with 50 mL of 23 mM ouabain in phosphate buffered saline. This eluate represented the final reagent and was dialyzed against 6 \times 4 L of phosphate buffered saline at 4 $^\circ$ C.

(B) Digoxin Assay

Measurement of digoxin in the test sample was performed in the following manner: A ouabain-affinity column as described in Example I (0.5 cm \times 4 cm) was loaded with a solution of Fab'- β -galactosidase conjugate and then washed with 50 mL of phosphate buffered saline. Samples (200 μ L) of human serum containing various amounts of digoxin were eluted through the column followed by 1 mL of phosphate buffered saline. The flow rate was approximately 0.5 mL per minute. The β -galactosidase activity in the eluates was determined spectrophotometrically upon the addition of the substrate o-nitrophenylgalactoside to a final concentration of 7.5 mM at 37 $^\circ$ C. The enzyme activity (A_{405} per min.) is plotted in FIG. 2 as a function of digoxin concentration in the 200 μ L samples.

I claim:

1. A method for determining the amount of an analyte in a liquid sample, comprising the following steps:

- (1) contacting a liquid sample suspected of containing analyte with a solid phase having immobilized thereon an analyte-analogue to which there is displaceably bound a labeled, anti-analyte antibody in molar excess over the analyte, wherein the dissociation constant between said antibody and the analyte-analogue is greater than the dissociation constant between said antibody and the analyte, whereby said antibody is displaced from the immobilized analyte-analogue as said antibody forms a complex with the analyte from the liquid sample;